

## **SUPPLEMENTARY MATERIAL**

### **Biodiversity of bacteriophages: morphological and biological properties of a large group of phages isolated from urban sewage**

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Table S1. The comparison of head diameter for phages of different families in the collection, MPS1 and MPS2

Phages	Phage family	Number of phages with head diameter:						
		30-40 nm	41-50 nm	51-60 nm	61-70 nm	71-100 nm	> 100 nm	
Collection	<i>Myoviridae</i>	1	0	1	11	12	0	25
Collection	<i>Siphoviridae</i>	0	9	19	6	1	0	35
Collection	<i>Podoviridae</i>	0	13	7	3	0	0	23
MPS1	<i>Myoviridae</i>	0	4	4	2	3	2	15
MPS1	<i>Siphoviridae</i>	1	31	29	10	8	0	79
MPS1	<i>Podoviridae</i>	0	0	4	2	0	0	6
MPS2	<i>Myoviridae</i>	0	0	5	12	7	12	36
MPS2	<i>Siphoviridae</i>	0	1	29	14	17	1	62
MPS2	<i>Podoviridae</i>	0	0	0	0	2	0	2

Table S2. The comparison of tail length of phages from different families in the collection, MPS1 and MPS2

Phages	Phage family	Number of phages with tail length:					
		9-39 nm	50-100 nm	100-200 nm	200-300 nm	> 300 nm	Sum
Collection	<i>Myoviridae</i>	0	4	21	0	0	25
Collection	<i>Siphoviridae</i>	0	0	35	0	0	35
Collection	<i>Podoviridae</i>	23	0	0	0	0	23
MPS1	<i>Myoviridae</i>	0	3	12	0	0	15
MPS1	<i>Siphoviridae</i>	0	10	35	20	14	79
MPS1	<i>Podoviridae</i>	6	0	0	0	0	6
MPS2	<i>Myoviridae</i>	0	4	27	5	0	36
MPS2	<i>Siphoviridae</i>	0	2	24	20	16	62
MPS2	<i>Podoviridae</i>	2	0	0	0	0	2

Table S3. Plaque diameter of phages from the collection, belonging to different families

Plaque diameter (mm)	Number of phages		
	<i>Myoviridae</i>	<i>Siphoviridae</i>	<i>Podoviridae</i>
≤1	19	3	0
2	6	5	4
3-6	0	27	15
5-7	0	0	4

Table S4. Bacterial strains used in the study

Bacterial species/serovars	Source or reference	Isolation material or other characteristics	Antibiotic resistance
<i>Pseudomonas aeruginosa</i> 436/1996	National Medicines Institute in Warsaw (Poland)	patient with cystic fibrosis	gentamicin-resistant
<i>Pseudomonas aeruginosa</i> 705/1996	National Medicines Institute in Warsaw (Poland)	patient with cystic fibrosis	-
<i>Pseudomonas aeruginosa</i> 708/1996	National Medicines Institute in Warsaw (Poland)	patient with cystic fibrosis	gentamicin-resistant
<i>Pseudomonas aeruginosa</i> 954/1996	National Medicines Institute in Warsaw (Poland)	patient with cystic fibrosis	gentamicin and tobramycin-resistant
<i>Pseudomonas aeruginosa</i> 961/1996	National Medicines Institute in Warsaw (Poland)	patient with cystic fibrosis	gentamicin and tobramycin-resistant
<i>Pseudomonas aeruginosa</i> 781/2003	National Medicines Institute in Warsaw (Poland)	wound	gentamicin and tobramycin-resistant
<i>Pseudomonas aeruginosa</i> 1000/2003	National Medicines Institute in Warsaw (Poland)	wound	gentamicin and tobramycin-resistant
<i>Pseudomonas aeruginosa</i> 1947/2003	National Medicines Institute in Warsaw (Poland)	wound	gentamicin and tobramycin-resistant
<i>Pseudomonas aeruginosa</i> 2317/2003	National Medicines Institute in Warsaw (Poland)	wound	gentamicin and tobramycin-resistant
<i>Pseudomonas aeruginosa</i> 2838/2003	National Medicines Institute in Warsaw (Poland)	wound	gentamicin and tobramycin-resistant
<i>Pseudomonas aeruginosa</i> 2222/2003	National Medicines Institute in Warsaw (Poland)	decubitus ulcers	gentamicin and tobramycin-resistant
<i>Pseudomonas aeruginosa</i> 1864/2003	National Medicines Institute in Warsaw (Poland)	wound	-
<i>Pseudomonas aeruginosa</i> 1872/2003	National Medicines Institute in Warsaw (Poland)	post-operative wound	gentamicin and tobramycin-resistant
<i>Pseudomonas aeruginosa</i> 886/2003	National Medicines Institute in Warsaw (Poland)	post-operative wound	gentamicin-resistant
<i>Pseudomonas aeruginosa</i> 2652/2003	National Medicines Institute in Warsaw (Poland)	post-operative wound	gentamicin and tobramycin-resistant
<i>Pseudomonas aeruginosa</i> 1369/2003	National Medicines Institute in Warsaw (Poland)	wound	gentamicin and tobramycin-resistant
<i>Pseudomonas aeruginosa</i> 1900/2003	National Medicines Institute in Warsaw (Poland)	suppuration	gentamicin and tobramycin-resistant
<i>Pseudomonas aeruginosa</i> 2262/2003	National Medicines Institute in Warsaw (Poland)	post-operative wound	gentamicin and tobramycin-resistant

<i>Pseudomonas aeruginosa</i> 2734/2003	National Medicines Institute in Warsaw (Poland)	post-operative wound	gentamicin-resistant
<i>Pseudomonas aeruginosa</i> 575/2003	National Medicines Institute in Warsaw (Poland)	decubitus ulcers	gentamicin-resistant
<i>Pseudomonas aeruginosa</i> 1368/2003	National Medicines Institute in Warsaw (Poland)	decubitus ulcers	gentamicin-resistant
<i>Pseudomonas aeruginosa</i> 1702/2009	National Medicines Institute in Warsaw (Poland)	bronchial mucus	-
<i>Pseudomonas aeruginosa</i> KBM	Department of Molecular Biology of University of Gdańsk (Poland)	wild type <i>P. aeruginosa</i>	-
<i>Staphylococcus aureus</i> 1893/2005	National Medicines Institute in Warsaw (Poland)	-	MRSA
<i>Staphylococcus aureus</i> 1391/2005	National Medicines Institute in Warsaw (Poland)	-	MRSA
<i>Staphylococcus aureus</i> 1932/2005	National Medicines Institute in Warsaw (Poland)	-	MRSA
<i>Staphylococcus aureus</i> 1781/2005	National Medicines Institute in Warsaw (Poland)	-	MRSA
<i>Staphylococcus aureus</i> 5069/2008	National Medicines Institute in Warsaw (Poland)	-	MRSA
<i>Staphylococcus aureus</i> 5074/2008	National Medicines Institute in Warsaw (Poland)	-	MSSA
<i>Staphylococcus aureus</i> 1881/2005	National Medicines Institute in Warsaw (Poland)	-	MSSA
<i>Staphylococcus aureus</i> 842/2005	National Medicines Institute in Warsaw (Poland)	-	MSSA
<i>Staphylococcus aureus</i> 421/2005	National Medicines Institute in Warsaw (Poland)	-	MSSA
<i>Staphylococcus aureus</i> 1088/2005	National Medicines Institute in Warsaw (Poland)	-	MSSA
<i>Staphylococcus aureus</i> 1899/1996	National Medicines Institute in Warsaw (Poland)	-	MSSA
<i>Staphylococcus aureus</i> 2977/2000	National Medicines Institute in Warsaw (Poland)	-	MSSA
<i>Staphylococcus aureus</i> 2233/2000	National Medicines Institute in Warsaw (Poland)	-	MSSA
<i>Staphylococcus aureus</i> 899/2003	National Medicines Institute in Warsaw (Poland)	-	MSSA
<i>Staphylococcus aureus</i> 303/2000	National Medicines Institute in Warsaw (Poland)	-	MSSA

<i>Staphylococcus aureus</i> 1365/2003	National Medicines Institute in Warsaw (Poland)	-	MSSA
<i>Staphylococcus aureus</i> 1562	Laboratory of Molecular Diagnostics of Intercollegiate Faculty of Biotechnology UG&MUG (Poland)	-	MRSA
<i>Staphylococcus aureus</i> 3659	Laboratory of Molecular Diagnostics of Intercollegiate Faculty of Biotechnology UG&MUG (Poland)	-	MRSA
<i>Staphylococcus aureus</i> 3578	Laboratory of Molecular Diagnostics of Intercollegiate Faculty of Biotechnology UG&MUG (Poland)	-	MRSA
<i>Staphylococcus aureus</i> 2002	Laboratory of Molecular Diagnostics of Intercollegiate Faculty of Biotechnology UG&MUG (Poland)	-	MRSA
<i>Staphylococcus aureus</i> 6504	Laboratory of Molecular Diagnostics of Intercollegiate Faculty of Biotechnology UG&MUG (Poland)	-	MRSA
<i>Staphylococcus aureus</i> 56/AS	Laboratory of Molecular Diagnostics of Intercollegiate Faculty of Biotechnology UG&MUG (Poland)	-	MRSA
<i>Staphylococcus aureus</i> 3442/1	Laboratory of Molecular Diagnostics of Intercollegiate Faculty of Biotechnology UG&MUG (Poland)	-	MRSA
<i>Staphylococcus aureus</i> 3442/2	Laboratory of Molecular Diagnostics of Intercollegiate Faculty of Biotechnology UG&MUG (Poland)	-	MRSA
<i>Staphylococcus aureus</i> 403/k/7	Laboratory of Molecular Diagnostics of Intercollegiate Faculty of Biotechnology UG&MUG (Poland)	community-aquired	MRSA
<i>Staphylococcus aureus</i> 264/k/7	Laboratory of Molecular Diagnostics of Intercollegiate Faculty of Biotechnology UG&MUG (Poland)	community-aquired	MRSA
<i>Staphylococcus aureus</i> 349/o/7	Laboratory of Molecular Diagnostics of Intercollegiate Faculty of Biotechnology UG&MUG (Poland)	community-aquired	MRSA
<i>Staphylococcus aureus</i> 345/o/7	Laboratory of Molecular Diagnostics of Intercollegiate Faculty of Biotechnology UG&MUG (Poland)	community-aquired	MRSA
<i>Staphylococcus aureus</i> 201/s/7	Laboratory of Molecular Diagnostics of Intercollegiate Faculty of Biotechnology UG&MUG (Poland)	community-aquired	MRSA
<i>Staphylococcus aureus</i> 887/s/7	Laboratory of Molecular Diagnostics of Intercollegiate Faculty of Biotechnology UG&MUG (Poland)	community-aquired	MRSA
<i>Staphylococcus aureus</i> 127/0/s/7	Laboratory of Molecular Diagnostics of Intercollegiate Faculty of Biotechnology UG&MUG (Poland)	community-aquired	MRSA
<i>Staphylococcus aureus</i> 621/s	Laboratory of Molecular Diagnostics of Intercollegiate Faculty of Biotechnology UG&MUG (Poland)	community-aquired	MRSA
<i>Staphylococcus aureus</i> 2997/s	Laboratory of Molecular Diagnostics of Intercollegiate Faculty of Biotechnology UG&MUG (Poland)	community-aquired	MRSA
<i>Staphylococcus aureus</i> 7-43/s/7	Laboratory of Molecular Diagnostics of Intercollegiate Faculty of Biotechnology UG&MUG (Poland)	community-aquired	MRSA

<i>Staphylococcus aureus</i> 103/k/7	Laboratory of Molecular Diagnostics of Intercollegiate Faculty of Biotechnology UG&MUG (Poland)	community-aquired	MRSA
<i>Staphylococcus sciuri</i> IO	Institute of Oceanology of Polish Academy of Sciences in Sopot (Poland)	urban sewage isolate	
<i>Salmonella enterica</i> Anatum	National Salmonella Centre at Medical University of Gdansk (Poland)	-	
<i>Salmonella enterica</i> Heidelberg	National Salmonella Centre at Medical University of Gdansk (Poland)	-	
<i>Salmonella enterica</i> Panama	National Salmonella Centre at Medical University of Gdansk (Poland)	-	
<i>Salmonella enterica</i> Reading	National Salmonella Centre at Medical University of Gdansk (Poland)	-	
<i>Salmonella enterica</i> London	National Salmonella Centre at Medical University of Gdansk (Poland)	-	
<i>Salmonella enterica</i> Tennessee	National Salmonella Centre at Medical University of Gdansk (Poland)	-	
<i>Enterococcus faecalis</i> 227	Department of Water and Waste-Water Technology of Gdansk University of Technology	urban sewage isolate	VRE
<i>Enterococcus faecalis</i> 230	Department of Water and Waste-Water Technology of Gdansk University of Technology	urban sewage isolate	VRE
<i>Enterococcus hirae</i> 238	Department of Water and Waste-Water Technology of Gdansk University of Technology	urban sewage isolate	-
<i>Enterococcus faecalis</i> 405	Department of Water and Waste-Water Technology of Gdansk University of Technology	urban sewage isolate	-
<i>Enterococcus faecalis</i> 406	Department of Water and Waste-Water Technology of Gdansk University of Technology	urban sewage isolate	-
<i>Enterococcus faecalis</i> 423	Department of Water and Waste-Water Technology of Gdansk University of Technology	urban sewage isolate	VRE
<i>Enterococcus faecium</i> 450	Department of Water and Waste-Water Technology of Gdansk University of Technology	urban sewage isolate	-
<i>Enterococcus faecalis</i> 546	Department of Water and Waste-Water Technology of Gdansk University of Technology	urban sewage isolate	-
<i>Enterococcus faecium</i> 549	Department of Water and Waste-Water Technology of Gdansk University of Technology	urban sewage isolate	-
<i>Escherichia coli</i> MG1655 CGSC#6300	Blattner <i>et al.</i> , 1997	-	-
<i>Escherichia coli</i> Tap90	Patterson & Dean, 1987	-	-

<i>Escherichia coli</i> Hfr3000	Bachmann, 1972	-	-
<i>Escherichia coli</i> EHEC O157:H7 ST2-8624	Griffin <i>et al.</i> , 1988	stool; Stx1 & Stx2	-
<i>Escherichia coli</i> EHEC O157:H7 CB571	Beutin <i>et al.</i> , 1989	stool ; Stx1 & Stx2	-
<i>Escherichia coli</i> EHEC O157:H7 EDL933	Beutin <i>et al.</i> , 1989; Perna <i>et al.</i> , 2001	stool; Stx1 & Stx2	-
<i>Escherichia coli</i> EPEC-A 129	Specialist Hospital of St. Wojciech in Gdansk (Poland)	stool ; EspA	-
<i>Escherichia coli</i> EPEC-B 21950	Specialist Hospital of St. Wojciech in Gdansk (Poland)	stool; EspB	-
<i>Escherichia coli</i> EPEC-C 22032	Specialist Hospital of St. Wojciech in Gdansk (Poland)	stool, EspC	-

MRSA – Methicillin-Resistant *S. aureus*, MSSA – Methicillin-Sensitive *S. aureus*, CA – Community-Aquired *S. aureus*, VRE – Vancomycin-resistant *Enterococcus*, EHEC – Enterohemorrhagic *E. coli*, EPEC – Enteropathogenic *E. coli*, Stx – Shiga toxin, Esp – *E. coli* secretion protein

1. Blattner F. R., Plunkett G. 3rd, Bloch C. A., Perna N. T., Burland V., Riley M., Collado-Vides J., Glasner J. D., Rode C. K., Mayhew G. F., Gregor J., Davis N. W., Kirkpatrick H. A., Goeden M. A., Rose D. J., Mau B. & Shao Y. The complete genome sequence of *Escherichia coli* K-12. *Science* **277**, 1453-1462 (1997).
2. Patterson T. A., & Dean M. Preparation of high titer lambda phage lysates. *Nucleic Acids Res.* **15**, 6298 (1987).
3. Bachmann B. J. Pedigrees of some mutant strains of *Escherichia coli* K-12. *Bacteriol Rev.* **36**, 525-557 (1972).
4. Griffin, P. M., Ostroff, S. M., Tauxe, R. V., Greene, K. D., Wells, J. G., Lewis, J. H. & Blake P. A. Illnesses associated with *Escherichia coli* O157:H7 infections. A broad clinical spectrum. *Ann Intern Med.* **109**, 705-12 (1988).
5. Beutin, L., Montenegro, M. A. & Orskov, I. Close association of verotoxin (Shiga-like toxin) production with enterohemolysin production in strains of *Escherichia coli*. *J. Clin. Microbiol.* **27**, 2559–2564 (1989).
6. Perna, N. T., Plunkett, G., Burland, V., Mau, B., Glasner, J. D., Rose, D. J., Mayhew, G. F., Evans, P. S., Gregor, J., Kirkpatrick, H. A., Pósfai, G., Hackett, J., Klink, S., Boutin, A., Shao, Y., Miller, L., Grotbeck, E. J., Davis, N. W., Lim, A., Dimalanta, E. T., Potamousis, K. D., Apodaca, J., Anantharaman, T. S., Lin, J., Yen, G. Schwartz, D. C., Welch, R. A. & Blattner, F. R. Genome sequence of enterohaemorrhagic *Escherichia coli* O157:H7. *Nature* **409**, 529-533 (2001).

**Figures S1-S5. BRIG-derived schematic maps of circular genomic comparisons between analyzed phages and reference phages selected from NCBI database as the most similar.** Image **S1** shows similarity between a central reference sequence of phage vB\_IME195 (green ring) and sequence of the analyzed phage vB\_Efae230P-4 (blue ring). Panel **S2** shows genomes of vB\_Pae575P-3 (blue ring) and vB\_Pae1369P-5 (red ring) compared against reference phage PA26 (green ring). Map **S3** presents comparison between vB\_Pae436M-8 (blue ring) and the most similar phage LMA2 (green ring). Picture **S4** shows similarity between phage vB\_SenM-2 (blue ring) and its reference phage Det7 (green ring). The last image **S5** presents genomic comparison between phages vB\_SscM-1 (blue ring), vB\_SscM-2 (red ring) and the most similar to them phage MCE-2014 (green ring). In each map, BLAST matches are colored on a sliding scale indicating a defined percentage identity of compared sequences at three levels: 50%, 70% and 100%. The innermost rings show genome locations and GC content (black). The most external rings show results of genome annotation process of the each analyzed phage. The color of annotations corresponds to the color of the analyzed phage genome.

Figure S1

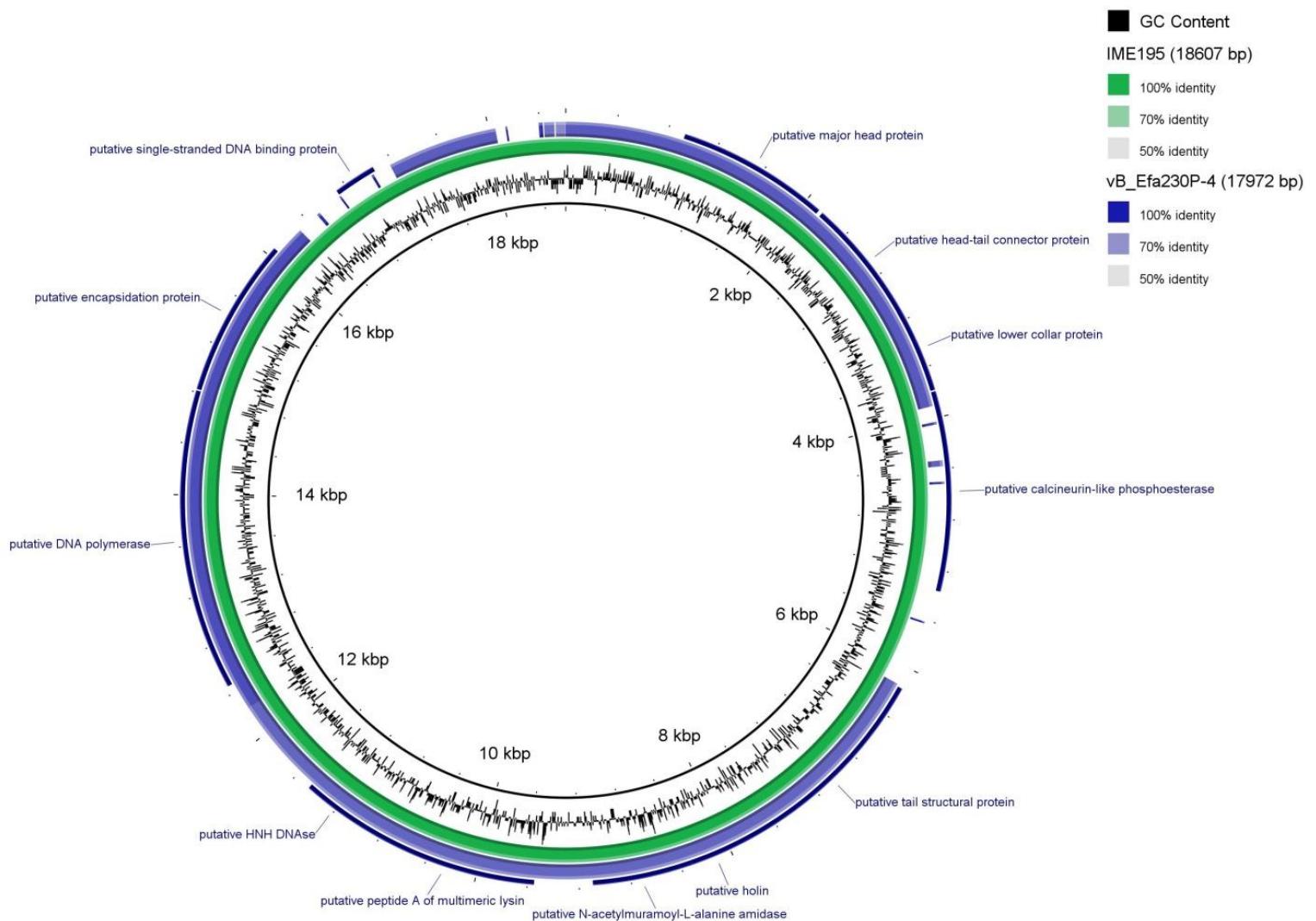


Figure S2

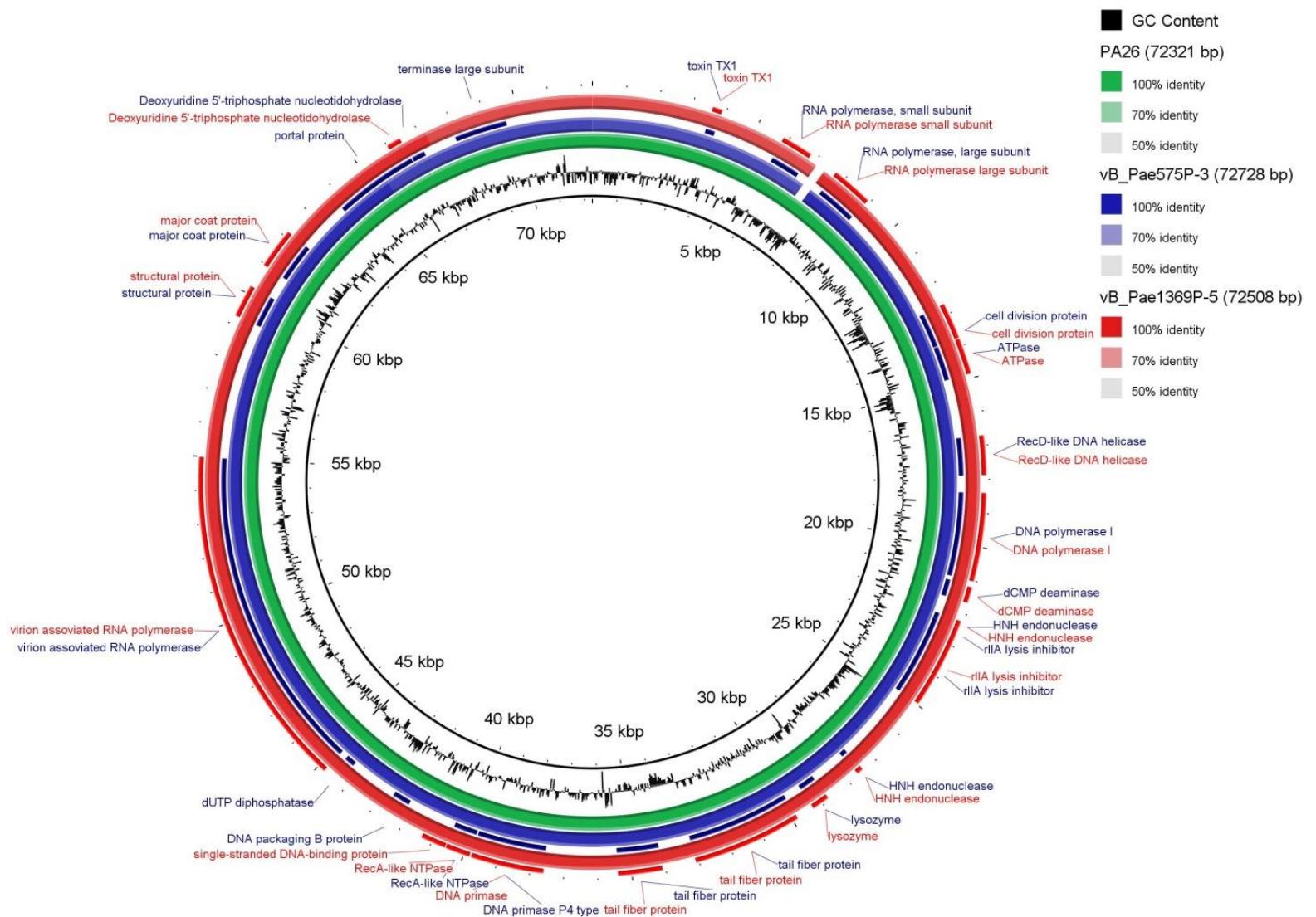


Figure S3

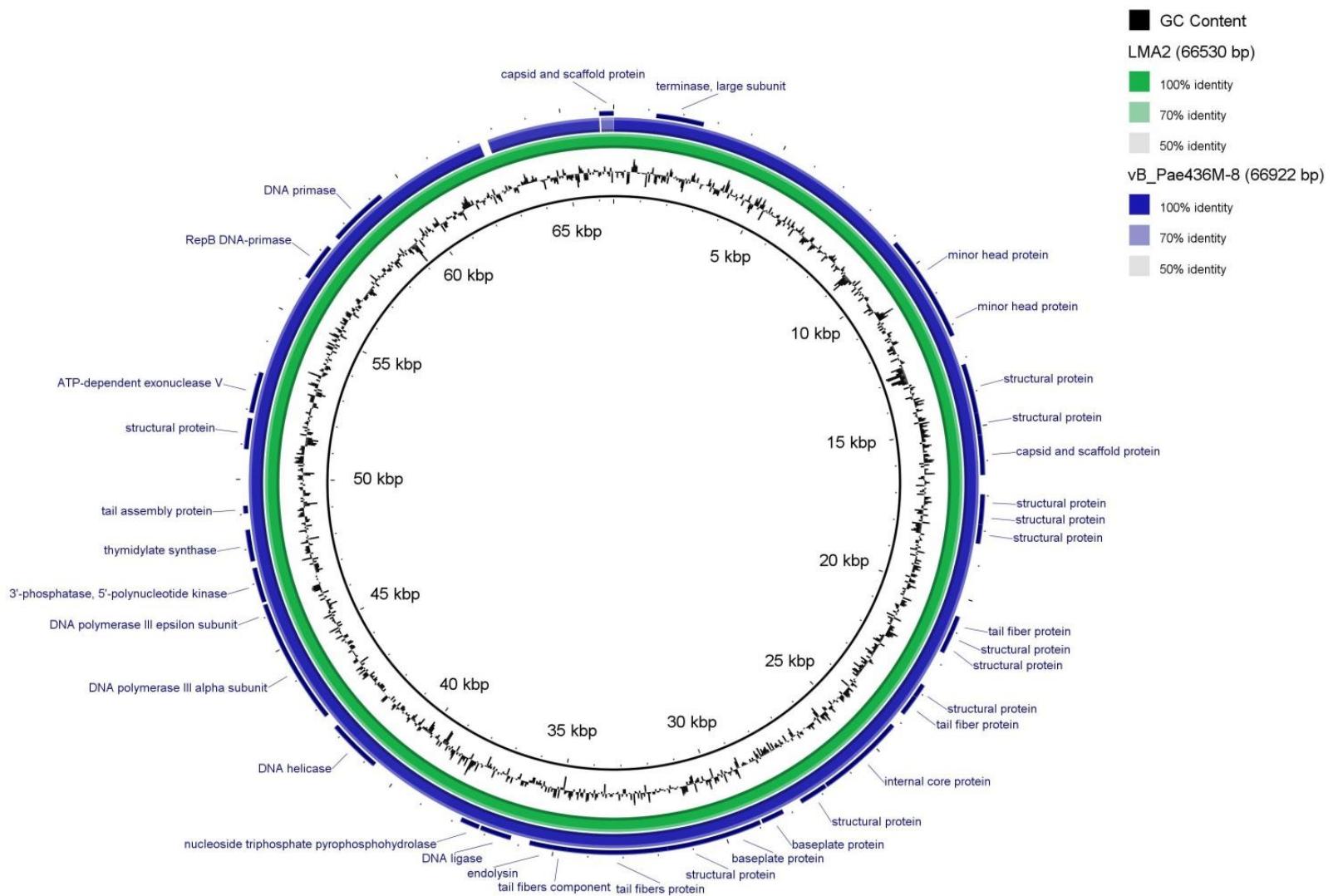


Figure S4

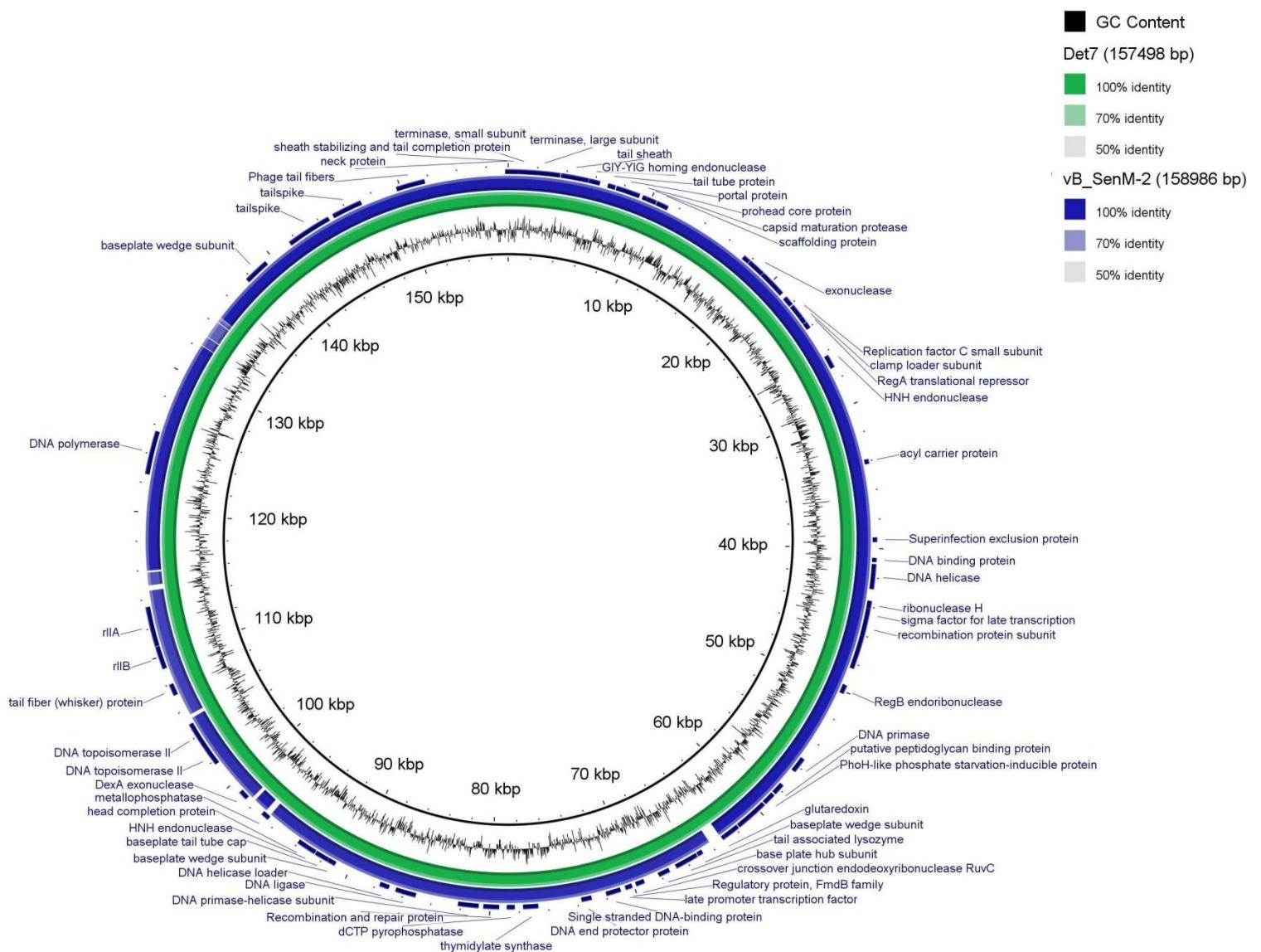


Figure S5

